protein in all mouse EOMs. By examining protein expression in wildtype vs. *Pitx2*^{null} EOM primordia, we found that *Pitx2* is epistatic to *Pax7*, *MyoD1* and *Myog* and are currently investigating *Myf5* and *Pax3*. We have identified predicted PITX2 binding sites in regulatory elements previously shown to recapitulate expression of *Myf5*, *MyoD1*, and *Myog* in EOMs, suggesting that PITX2 may directly regulate these genes. We are currently testing this hypothesis. These results indicate that *Pitx2* is an essential early component of the regulatory network leading to EOM specification.

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Fate mapping reveals abnormalities in brain region-specific neuronal differentiation and migration with loss of Pitx2 function

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PITX2, a paired-like transcription factor, is mutated in Rieger syndrome, an autosomal dominant haploinsufficiency disorder affecting eyes, teeth, and umbilicus, with occasional brain and pituitary defects. Prior studies identified abnormal gene expression and neuronal projections in the subthalamic nucleus and superior colliculus of *Pitx2* null mouse embryos but lacked a direct marker for mutant cells. Here, we report *Cre/loxP* fate mapping of *Pitx2* lineage neurons in the central nervous system, using a *Pitx2*^{cre} knock-in allele and a previously characterized transgenic β-actin conditional *lacZ* reporter strain *(N-lacZ)*. We observed a complete absence of *Pitx2* lineage neurons in the subthalamic nucleus of X-gal-stained whole mount or sectioned *Pitx2*$^{cre/-}$; *N-lacZ* embryos, indicating failure of normal differentiation and migration. *Lmx1b* neurons were also absent from the subthalamic nucleus, but preserved in medial areas of the neuroepithelium, suggesting that *Pitx2* deficiency does not completely abolish the *Lmx1b* lineage. We also observed a medial shift in neurons of the developing superior colliculus, which could be explained by a developmental delay or arrest in radial and/or tangential migration. These observations indicate that *Pitx2* promotes regionally specific neuronal migration and differentiation in the developing mouse brain, with potential effects on *Lmx1b* transcription. Ongoing studies will identify *Pitx2* cell fates in the embryonic and adult brain and explore the molecular mechanisms of regionally specific neuronal differentiation and migration.

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LHX3 regulation of pituitary size and cell specification

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The LIM homeodomain transcription factor LHX3 is essential in early pituitary development. Previous studies by Westphal and co-workers (Sheng et al., Science 272: 1004–7, 1996) established that *Lhx3*$^{-/-}$ mutants have profound pituitary hypoplasia and fail to specify four of the five hormone-producing cell types in mice. We have analyzed the *Lhx3* knock-out mice to establish the mechanism that underlies the pituitary phenotype. We found that LHX3 is necessary at e12.5 for expression of the critical transcription factors *Foxl2*, *Isl1*, and *Pitx2* but not for *Pitx1* or *T-pit*. At e14.5, we found that LHX3 is not necessary for expression of *Nr5a1 (SF1)*, an important transcription factor for gonadotroph cell differentiation. The expression of SF1 in Lhx3 mutants suggests that gonadotroph precursors are formed but are unable to complete their differentiation program. The pituitary hypoplasia in *Lhx3*-deficient mice is partly attributable to an increase in cell death in *Lhx3*$^{-/-}$ embryos at e12.5. In conclusion, our studies place *Lhx3* in the genetic hierarchy of transcription factors that regulate pituitary development and reveal a role for *Lhx3* in cell survival.

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Sox9 genes mediate Fgf-dependent otic induction

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The inner ear derives primarily from ectoderm that is competent to respond to otic induction signals. We previously suggested that zebrafish Dlx3b–Dlx4b may provide naïve ectoderm with otic competence to respond to Fgf3 and Fgf8. By implanting Fgf8-soaked beads into various ectodermal regions at mid-gastrula stage, we now show that Fgf8 is a potent ear inducer in the non-neural ectoderm. In Dlx3b–Dlx4b defective background, ear induction by Fgf8 beads diminishes. However, over-expression of *dlx3b–dlx4b* has not changed the ear induction by Fgf8 beads, likely because Dlx3b and Dlx4b are already distributed in most non-neural ectoderm. Sox9a and Sox9b lie genetically downstream of Dlx3b–Dlx4b in the Fgf-dependent pathway and Fgf8 beads induce both *sox9* gene expressions ectopically. Fgf-independent pathways mediated by Foxi1, Dlx3b–Dlx4b, and retinoid acid also regulate both genes. As a consequence, completely removing both Sox9 functions leads to a lack of ear development. Moreover, forced expression of either Sox9a or Sox9b induces ectopic *dlx3b* that leads to multiple ectopic otic tissues. Dlx3b–Dlx4b and Sox9 proteins are largely absent in the neural ectoderm during early stages of otic induction and we find that Fgf8 beads are unable to induce otic cells in neural tissues even when *dlx3b–dlx4b* is over-expressed. In contrast, Fgf8 beads can induce ectopic ear cells in the neural tube and spinal cord when *sox9b* is over-expressed. Therefore, we propose that Sox9 proteins integrate multiple genetic pathways to ensure that cells express the